

Production, Properties and Industrial Applications of Microbial Xylanase

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ABSTRACT:

Xylanase is produced from various microorganisms such as bacteria, fungi, actinomycetes and yeast in the presence of xylan rich agricultural residues. The essential factors for efficient production of xylanase are the appropriate medium composition and an inducing substrate. Recently, there has been much industrial interest of xylanase, as a supplement in animal feed, for the manufacture of bread, food and drinks, textiles, bleaching of cellulose pulp, ethanol and xylitol production. There have been various attempts to produce xylanase efficiently from inexpensive raw materials. This article reviews about the xylanase producing microorganisms, raw materials, mode of fermentation for xylanase production, properties of xylanase and various industrial applications with a particular focus on recent investigations.

Key words: Microbial xylanase, xylan, Extremophiles, Industrial application

INTRODUCTION

Xylanases are glycosidases [O-glycoside hydrolases, EC 3.2.1.8] which catalyze the endohydrolysis of 1,4- β -D-xylosidic linkages in xylan. They are a widespread group of enzymes, involved in the production of xylose, a primary carbon source for cell metabolism and in plant cell infection by plant pathogens, and are produced by a plethora of organisms including bacteria, algae, fungi, protozoa, gastropods and anthropods [1]. First reported in 1955 [2], they were originally termed pentosanases, and were recognized by the International Union of Biochemistry and Molecular Biology [IUBMB] in 1961 when they were assigned the enzyme code EC 3.2.1.8. Their official name is endo-1,4- β -xylanase, but commonly used synonymous terms include xylanase, endoxylanase, 1,4- β -D-xylan-xylanohydrolase, endo-1,4- β -D-xylanase, β -1,4-xylanase and β -xylanase.

Xylanases produced by microorganisms have attracted a great deal of attention during the past few decades because of their potential biotechnological applications in various industries, including the food, feed, fuel, textile, pharmaceutical, soap and pulp and paper industries, in waste treatment[3] fiber modification, extraction of coffee, starch industries and juice clarification [4]. Recently, the interest in cellulase- free xylanase has focused on pulp and bleaching processes due to the potential industrial use [5].The chemical bleaching process uses a large amount of chlorine and chlorine-based chemicals. The by-products formed during chemical processing are toxic, mutagenic, persistent, bioaccumulating, and cause numerous harmful disturbances in biological systems. The new bleaching procedures replace the classical process based on chlorine, chlorinated compounds, hydrogen peroxide, oxygen, ozone, etc., with another method called the biobleaching process [5,6,7]. This article presents a review of recent advances in the biotechnological production of xylanase, as well as its recent applications and properties.

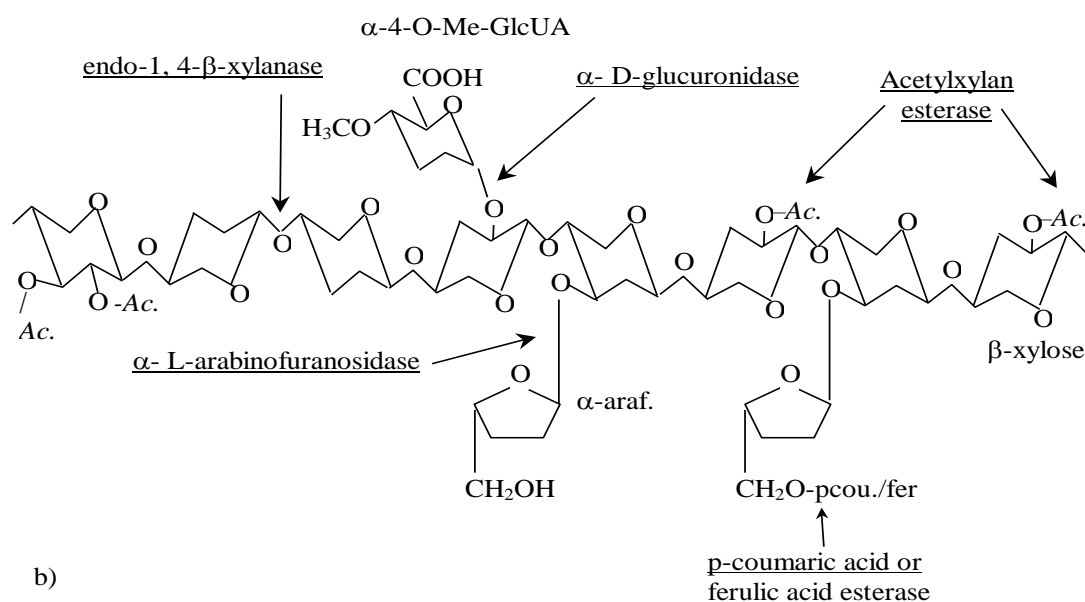
XYLAN

The substrate of xylanases, xylan, is a major structural polysaccharide in plant cells, and is the second most abundant polysaccharide in nature, accounting for approximately one-third of all renewable organic carbon on earth [8]. Due to its heterogeneity and complexity, the complete hydrolysis of xylan requires a large variety of cooperatively acting enzymes [4,9,10] [Fig. 1]. Endo-1,4- β -D-xylanases (EC 3.2.1.8) randomly cleave the xylan backbone, β -D-xylosidases (EC 3.2.1.37) liberate xylose monomers from the non-reducing end of xylo-oligosaccharides and xylobiose while removal of the side groups is catalysed by α -L-arabinofuranosidases (EC 3.2.1.55), α -D-glucuronidases (EC 3.2.1.139), acetylxyylan esterases (EC 3.1.1.72), ferulic acid esterases (EC 3.1.1.73) and p-coumaric acid esterases (EC 3.1.1.-). Indeed, complete xylanolytic enzyme systems, including all of these activities, have been found to be quite widespread among fungi [11,12], actinomycetes [13] and bacteria [12] and some of the most important xylanolytic enzyme producers include the *Aspergilli*, *Trichodermi*, *Streptomyces*, *Phanerochaetes*, *Chytridiomycetes*, *Ruminococci*, *Fibrobacteres*, *Clostridia* and *Bacilli* [10,12,14,15].

MICROBIAL XYLANASES

Xylanases have been produced in either solid-state fermentation [SSF] or submerged fermentation with the prevalence of the last one [16]. Most research has used submerged cultures, which allows control over the degree of aeration, pH and temperature of the medium, and control over other environmental factors required for the optimum growth of microorganisms. However, SSF has gained renewed interest in recent years for the production of many enzymes due to lower operation costs and energy requirements, and simpler plant and equipment projects when compared to submerged microbial cultures [17,18].

a)



b)

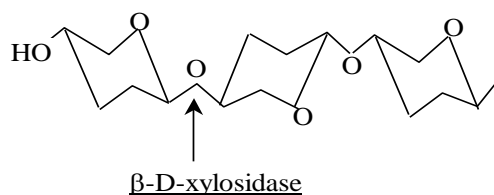


Fig. 1. (a) Structure of xylan and the sites of its attack by xylanolytic enzymes. The backbone of the substrate is composed of 1, 4-β-linked xylose residues. Ac., Acetyl group; α-araf., α-arabinofuranose; α-4-O-Me-GlcUA, α-4-O-methylglucuronic acid; pcou., *p*-coumaric acid; fer., ferulic acid. (b) Hydrolysis of xylo-oligosaccharide by β-xylosidase.

SSF is defined as the fermentation involving solid substrates with low moisture content. The substrate, however, must contain sufficient moisture to support the growth and metabolism of the microorganisms [18]. Solid-state fermentation is closer to natural system and has proved to be more efficient in producing certain enzymes and metabolites [19,20]. Filamentous fungi [21,22] and actinomycetes [23] prefer to grow well on moist substrates with less moisture content, whereas bacteria need high moisture contents and unable to grow in these conditions. There are only fewer reports related to successful utilization of bacteria for SSF [24,25]. Specific xylanases are synthesized when microorganisms are cultured on xylan, whereas on cellulose the organisms produce cellulases in association with xylanases, because the cellulose substrate contains traces of hemicelluloses.

In the textile and paper industries, it is important to be able to obtain xylanases free of cellulose activity, as it is necessary to extract hemicellulose from the natural fibres, without damaging the cellulose.

Polysaccharides, such as xylan, are known to induce the activity of enzyme complexes in microorganisms [26]. This effect was questioned by many researchers for some time, as these macromolecules are unable to make contact with sites within the cell, in order to influence the regulation of gene expression. For induction of the xylanolytic enzymes to occur, there has to be physical contact between part of the regulatory machinery of the cell and the inducer; which suggests the existence of some recognition site on the cell surface. Constitutive xylanases, at relatively low levels of activity, are supposed to be responsible for the initial hydrolysis of xylan, producing small β-D-xylopyranosyl oligosaccharides such as xylobiose and xylotriose, among others [4,27,28,]. Some authors then consider the xylobiose formed to be the true inducer of endoxylanase synthesis [29,30]. With the help of β-xyloside permeases [Fig. 2], these oligosaccharides are transported into the cell, where they trigger the expression of the xylanolytic system genes. The permease activity of the induced cells diminishes in the presence of glucose, but it is very

efficient in the presence of the xylanolytic inducers. [31,32,33,34].

The cost of an enzyme is one of the main factors determining the economics of a process. Reducing the costs of enzyme production by optimizing the fermentation medium and process is the goal of basic research for industrial applications in general; optimization by the traditional ‘one- factor-at-a-time’ technique was used. This method was determined by varying one factor while keeping the other factors at a constant level. This method, although simple, often requires a considerable amount of works and time. Recently, statistical designs for optimization have been successfully employed in enzyme production [35,36]. There have been numerous investigations on the development of biotechnological processes for

xylanase production, with the ultimate objectives to enable the process to be more efficient and economical.

Xylanases have been produced from a wide range of microorganisms including bacteria, fungi, and yeast as shown in Tables 1, Table 2, and Table 3, respectively. The xylanases activities shown in Tables 1, Table 2, and Table 3 are certainly not comparable, because of the large number of xylanase assay procedures and different conditions, e.g. temperature, duration of the incubation or substrate employed. Bacterial and fungal groups constitute the greatest variety of xylanase producers.

Table 1. Properties of xylanases from bacteria

Microorganism	Substrate	Growth Condition			Mode of operation	Enzyme Yield	Ref.
		Temp °C	pH	Duration			
<i>Bacillus pumilus</i>	wheat bran	30°C	7	5 days	SSF, 250 mL Erlenmeyer flasks, 150 rpm	5582 U/gds	[37]
<i>Cellulomonas flavigena</i>	xylan	30°C	7.3	56-72 h	SF, shake flask culture, 150 rpm	440 IU/L.h	[38]
<i>Pseudomonas sp.</i>	Wheat bran	37°C	7.2 to 8.0	24 h	250 mL Erlenmeyer flasks, 220 rpm	1245 U/mL	[39]
<i>Cellulomonas flavigena</i>	Sugar cane bagasse	37°C	7.0	24 h	250ml Erlenmeyer flask , 150 rpm	24 IU/mg	[40]
<i>Geobacillus thermoleovorans</i>	1% wheat bran	70°C	7.5	42 h	250mL Erlenmeyer flasks, 250 rpm		[41]
<i>Cellulomonas flarigena</i>	2% sugarcane bagasse	37°C	7.0	44 h	SSF, 750 mL jar fermentor, 750 rpm	4.2 IU/mL.h	[42]
<i>Micrococcus sp.</i> AR135	Xylose	32°C		48 h	500 mL baffled flasks	3.7 U/mL	[43]
<i>Bacillus sp.</i> 1-1018	5% Xylan	50°C	7.3	18 h	SF, 250 mL conical flasks, 250 rpm	-	[44]
<i>Bacillus species</i> [NCL-87-6-10, NCIM 2128]	3% Wheat bran	-	-	48 h	SF, shake flasks	100-120 IU/mL	[45]
<i>Bacillus sp.</i> [V1-4]	1% Xylan	32°C	9	2 days	SF, fermentor cultivation	-	[46]
<i>Dictyoglomus sp.</i> B ₁	0.15% beech xylan	70°C	8	4-6 days	SF [batch]	2312.0 U/L	[47]
	0.15 % beech xylan	70°C	8	4-6 days	1L infusion bottles, continuous stirred fermentor	783 U/L	
<i>Bacillus sp.</i> 41M-1	0.5% xylan	37°C	8	5 days	-	-	[48]
<i>Bacillus Circulans</i> VTT-E-87305	2% Beech xylan	-	8 to 8.5	2 days	CMF fermentors	6600 nKat/mL	[49]
<i>Bacillus thermoalkalo philus</i>	Rice husk bagasse	70°C	9.0	24 h	Shake-culture flasks, 200 rpm	-	[50]
<i>Bacillus sp.</i>	1% Xylan	45°C	10	48 h	L-Shape test tubes	-	[51]
<i>Arthrobacter sp.</i> MTCC 5214	Wheat bran	30°C	9	4 days	SSF, 500 mL Erlenmeyer flasks	-	[52]
<i>Bacillus circulans</i> BL53	Fibrous Soy residue	25°C	-	120 h	500 mL cylindrical glass bioreactor SSF	-	[53]
<i>Bacillus sp.</i> k-8	0.5% oat spelt xylan	37°C	10.5	2 days	200 rpm	-	[54]
<i>Bacillus Coagulans</i>	Birch wood [or] Wheat straw	45°C	7	-	Fermentor, 200-400 rpm	-	[55]
<i>Clostridial species</i>	% oat spelt xylan	37°C	7	21-24 h	-	-	[56]
<i>Bacillus circulans</i> BL53	Fibrous Soy residue	37°C	-	96 h	Horizontal rotated drum bioreactor	-	[57]
<i>Bacillus Coagulans</i> BL69	Fibrous Soy residue	37°C	-	72 h	SSF, 500mL cylindrical bioreactor	-	[58]
<i>Acrophialophora nainiana</i>	1% Oat spelt Xyln	40°C	-	7 days	Erlenmeyer flasks, 100rpm	-	[59]
<i>Cellomonas flavigena</i>	1% sugarcane bagasse	-	-	-	Bench reactor	-	[60]
<i>Bacillus circulans</i> D1	Xylan	45°C	-	72 h	SF, 125mL Erlenmeyer flasks, 150 rpm	-	[61]
<i>Cellulomonas flavigena</i>	sugarcane bagasse	37°C	-	48 h	150 rpm	-	[62]

SF- Submerged Fermentation; SSF- Solid State Fermentation

Table 2. Properties of xylanases from fungi

Microorganism	Substrate	Growth Condition			Mode of Operation	Enzyme Yield	Ref.
		Temp °C	pH	Duration			
<i>Aspergillus awamori</i>	1% apple pectin [or] 1% birchwood xylan	30°C	-	-	SSF	-	[63]
<i>Aspergillus niger</i> B03	1.5% wheat bran 2.4% corn cobs 0.6% malt sprout	28°C	6.7 to 6.8	64 h	SF, 50/500 mL flasks, 180 rpm	996.30 U/mL	[64]
<i>Trichoderma longibrachiatum</i>	Wheat straw	26°C	5.5	3 days	SSF, 10/250 mL flasks	417.2 U/g	[65]
<i>Trichoderma harzianum</i>	1% xylan	30°C	-	7 days	150 rpm	46.3 U/mg	[66]
<i>Polyporus squamosus</i>	1% Sugar beet	28°C	5.5	7 days	SSF, 300mL Erlenmeyer flasks	0.173 U/mL	[67]
<i>Penicillium citrinum</i>	1% Birch-wood xylan	30°C	7.5	3 days	500mL Erlenmeyer flasks, 150 rpm	3.5 U/mL	[68]
<i>Aspergillus niger</i>	0.3% xylan from corn cob.	28°C	-	96 h	20L fermentor, 300 rpm, Aeration rate 1.5 vvm	-	[69]
<i>Aspergillus foetidus</i>	1% Birchwood xylan	30°C	5.0	7 days	SF, 100/500mL Erlenmeyer flasks, 180 rpm	80.5 U/mL	[70]
<i>Trichoderma harzianum</i>	1% xylan	30°C	-	7 days	150 rpm	-	[71]
<i>Aspergillus Caespitosus</i>	1.0% Sugarcane bagasse	40°C	8.0	48 h	125mL Erlenmeyer flasks, 100 rpm	-	[72]
<i>Aspergillus Versicolor</i>	1% wheat bran	30°C	5.8 or 6.5	5 or 9 days	50/250 mL Erlenmeyer flasks	-	[73]
<i>Aspergillus japonicus</i>	1% wheat bran	25°C	5.0	120 h	250mL Erlenmeyer flasks, 125 rpm	177.9 U/mL	[74]
<i>Myceliophthora sp.</i> 1M1 387099	Rice straw, wheat straw, bagasse, Corn cob and wheat bran	45°C	5.0	5 days	-	-	[75]
<i>Trichoderma reesei</i> Rut C-30	xylan	28°C	4 to 6.5	5 days	2L glass vessel bio- reactors, 400 rpm, Aeration 1 vvm	-	[76]
<i>Thermoascus aurantiacus</i> ATCC 204492	5% Sugarcane bagasse	45°C	-	10 days	SSF, Glass column reactor.	1597 U/g	[77]
<i>Pleurotus Ostreatus</i> SYJ042	2.5% Corn cob 2.5% Wheat bran	26°C	6.0	7 days	50/150 mL flasks, 160 rpm	24.98 U/mL	[78]
<i>Aspergillus fumigatus</i> AR1	1% Simple sugars 1% Agricultural Residues	30°C	5.0 to 9.0	60 h	50/250 mL Erlenmeyer flasks, 160 rpm	135 U/mL 30 u/mL	[79]
<i>Aspergillus nidulans</i>	3% Corn cob	30°C	-	6 days	50/250 mL Erlenmeyer flasks, 120 rpm	220 U/mL	[80]
<i>Aspergillus niger</i> An-76	2% wheat bran	30°C	6.0	72 h	150 rpm	-	[81]
<i>Aspergillus nidulans</i> KK-99	2% wheat bran	37°C	10	144 h	Shaking flasks, 200 rpm	40 IU/mL	[82]
<i>Aspergillus niveus</i> RS2	0.5% oat spelt xylan	45°C	8	5 days	100/250 mL Erlenmeyer flasks, 180 rpm	18.2 U/mL	[83]
<i>Penicillium brasilianum</i> IBT 20888	0.2% [BSG] wheat bran Sugar beet pulp straw, and corn cobs	30°C	6	9 days	100 mL Erlenmeyer flasks, SSF	709 U/g BSG	[84]
<i>Trichoderma reesei</i> Rut C-30	L- arabinose	28°C	6	-	2 L Bioreactor, 300-400 rpm, gas flow 0.5 vvm	-	[85]
<i>Aspergillus phoenicis</i>	1% xylan birch wood	25°C [or] 42°C	-	72 h	250 mL Erlenmeyer flasks, 100 rpm	-	[86]
<i>Thermoascus aurantiacus</i>	4.02% Solka Floc	47°C	-	-	3.5 L/5 L Bio reactor, Aeration rate 0.5 vvm, 200- 250 rpm	-	[87]
<i>Aspergillus tamarii</i>	1% oat spelt xylan	30°C	-	5 days	50/250 mL conical flasks, 120 rpm	100 U/mL	[88]
<i>Trichoderma Reesei</i>	0.3% Solka Floc	27°C	-	-	100/500 mL Erlenmeyer flasks, 150 rpm	-	[89]
<i>Cyathus Stercoreus</i>	0.5% xylan	30°C	5.6	9-12 days	-	725 U/L	[90]
<i>Aspergillus nidulans</i> CECT 2455	1% Oat spelt xylan	37°C	-	32 h	200/1000 mL flasks, 200 rpm	-	[91]
<i>Phanerochaete chrysosporium</i>	0.5% Oat spelt xylan	37°C	-	8 days	Erlenmeyer flasks, 200 rpm	-	[92]
<i>Thermomyces Lanuginosus</i>	3.25% corn cobs	50°C	7.5	118 h	100/300 mL Erlenmeyer flasks, 50 rpm	32500 n kat/mL	[93]
<i>Thermomyces Lanuginosus</i> DSM5826	3% Corn cobs	50°C	6.5	7 days	100/300 mL Erlenmeyer flasks, 150 rpm	1438.7 U/mL	[94]
<i>Thermomyces lanuginosus</i> CBS 288.54	2% Corn cob Xylan	50°C	7.0	7 days	100/30 mL Erlenmeyer flasks, 150 rpm	-	[95]
<i>Trichoderma reesei</i>	[1%-8%] Solka-floc, Canola meal, larch wood xylan	27°C	-	9-12 days	200/500 mL Erlenmeyer flasks, 200 rpm	210 IU/mL	[96]

SF- Submerged Fermentation; SSF- Solid State Fermentation

Table 3. Properties of xylanases from yeast

Microorganism	Substrate	Growth Condition			Mode of operation	Enzyme Yield	Ref.
		Temp °C	pH	Duration			
<i>Thermomyces lanuginosus</i> ATCC 36350	Bagasse pulp	45°C	7.0	4 days	SSF, 500mL Erlenmeyer flasks	5098 U/g	[97]
<i>Streptomyces sp.</i> Ab106	Sugarcane bagasse	50°C	7.0	-	5l fermentor, 150rpm, Aeration rate 1 vvm	16 IU/mL	[98]
<i>Streptomyces sp.</i> Ab106	1% Sugarcane bagasse	50°C	7.0	5 days	250 / 1000mL Erlenmeyer flasks, 100 rpm	15 IU/mL	[99]
<i>Streptomyces sp.</i>	2% Rice strain pulp	28°C	7.0 to 7.2	5 days	50/250 mL flasks, 200 rpm	43.01 U/mL	[100]
<i>Streptomyces sp.</i> AMT-3	1% larch wood xylan	30°C	7.0	10 days	200ml/1000mL Erlenmeyer flasks, 150 rpm	70.0 U/mL	[101]
<i>Cryptococcus adeliae</i>	1% xylan	4°C	7.5	8 days	Shake flasks	400 nkat/mL	[102]
<i>Streptomyces sp.</i> QG-11-3	1% wheat bran	37°C	-	96 h	50/250 mL baffled flasks, 200 rpm	96 IU/mL	[103]
Yeast strain NCIM 3574	4% xylan	28°C	3.5 to 5.5	72 h	50/250 mL Erlenmeyer flasks, 200 rpm	570 IU/mL	[104]

SF- Submerged Fermentation; SSF- Solid State Fermentation

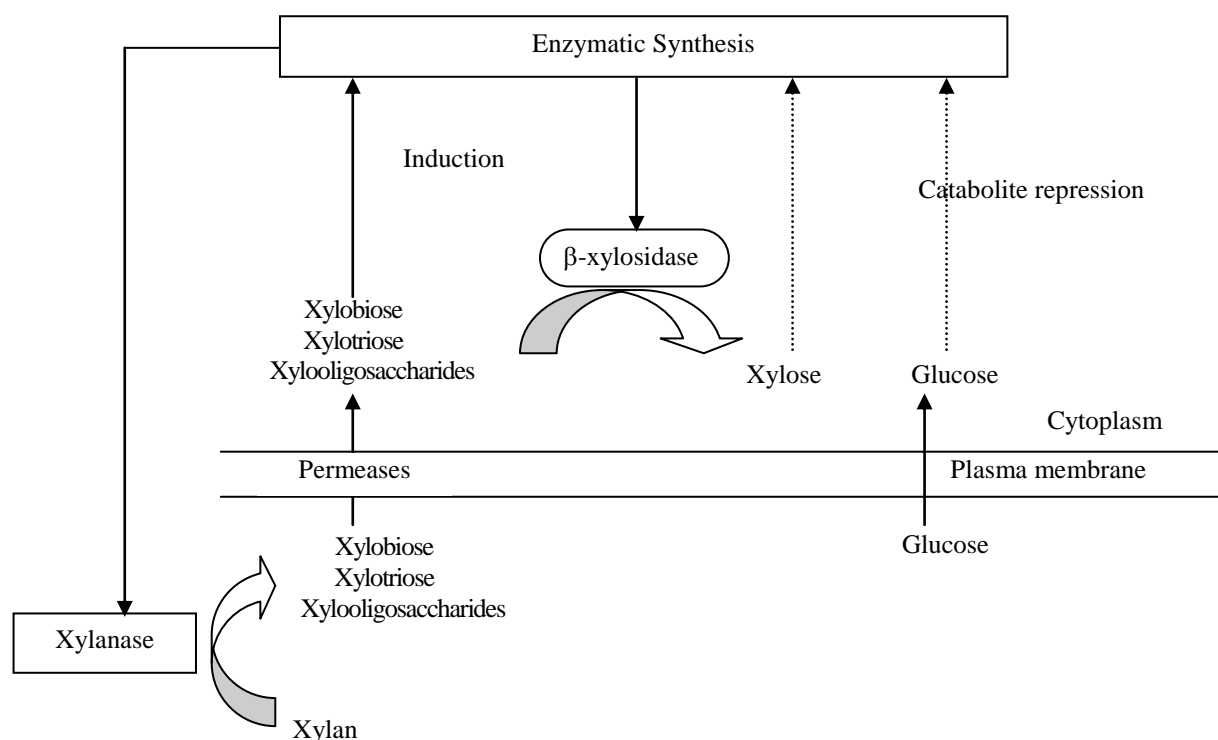


Fig. 2. Hypothetical scheme of the regulation of the xylanolytic complex involving endoxylanase and β -xylosidase. Constitutive endoxylanases degrade xylan to the xylooligosaccharides that through permeases enter the cytoplasm uncoupling the transcription of genes responsible for the production of endoxylanases and β -xylosidases.

Bacteria such as *Bacillus pumilus* [37], *Cellulomonas flavigena* [38], *Pseudomonas sp.*, [39] *Bacillus sp.* NCL-87-6-10, NCIM 2128, [45] and *Bacillus circulans* VTT-E-87305 [49] produced abundant xylanase, as summarized in Table 1. In fungi [Table 2], *Aspergillus niger* BO3 [64], *Trichoderma longibrachiatum* [65], *Aspergillus japonicus* [74], *Thermoascus aurantiacus* ATCC 204492 [77], *Aspergillus nidulans* [80], *Penicillium brasilianum* IBT 20888 [84], *Cyathus stercoreus* [90], *Thermomyces lanuginosus* DSM 5826 [94] and

Trichoderma reesei [95] produced high amount of xylanase.

Xylanase produced by yeast is shown in Table-3. *Thermomyces lanuginosus* ATCC 36350 [96], *Streptomyces sp.* QG-11-3 [101] *Cryptococcus adeliae* [102], *Streptomyces sp.* AMT-3 [103] and yeast strain NCIM3574 [104] produced high amount of xylanase. Alternatively, genetically engineered organisms could be used to produce xylanases exclusively. However, the back mutation of genetically engineered

microorganisms is the main problem preventing industrial use [105,106]. On the other hand, it is possible to isolate naturally occurring microorganisms which produce totally cellulase- free xylanases.

EXTREMOPHILIC XYLANASES

The preponderance of xylanases studied are of fungal or bacterial origin and in the majority of cases are found to be optimally active at, or near mesophilic temperatures [approximately 40–60 °C] [10, 12] and neutral [in particular for bacterial xylanases] or slightly acidic [in particular for fungal xylanases] pH. Nevertheless, xylanases have also been reported which are not only stable, but active, at the extremes of pH and temperature. Indeed, xylanases active at temperatures ranging from 5 to 105 °C [107,108,109] pHs from 2 to 11 [107,110,111] and NaCl concentrations as high as 30% [112,113] have been reported. These are produced by micro-organisms which have colonized environments that may be said to be extreme from an anthropocentric point of view and which produce enzymes adapted to these extreme habitats. Of the extremophilic xylanases, the thermophiles, alkaliphiles and acidophiles have been the most extensively studied while cold-adapted xylanases have been much less investigated.

THERMOPHILES FOR XYLANASE PRODUCTION

A number of thermophilic [optimal growth at 50–80°C] and hyperthermophilic [optimal growth at >80°C] xylanase producing micro-organisms have been isolated from a variety of sources, including terrestrial and marine solfataric fields, thermal springs, hot pools and self-heating decaying organic debris [114-119]. Xylanases have been isolated from various thermophilic and hyperthermophilic organisms, including *Thermotoga* sp. [120,121], *Cellulosiruptor* sp. [122], *Rhodothermus marinus* [123], *Bacillus stearothermophilus* [124], *Thermoascus aurantiacus* [125] and *Clostridium thermocellum* [126]. Family 11 thermophilic xylanases have also been isolated from *Thermomyces lanuginosus* [114,127], *Paecilomyces varioti* [128], *Caldicellulosiruptor* sp Rt69B.1. [129], *Dictyoglomus thermophilum* [130], *Chaetomium thermophilum* [131], *Nonomuraea flexuosa* [131] and *Bacillus* strain D3 [132,133].

Those from *Nonomuraea flexuosa* and *Dictyoglomus thermophilum* [130] are among the most stable, with apparent temperature optima of 80 and 85°C, respectively. In addition to the above mentioned xylanase producing bacteria a number of xylanase producing hyperthermophilic archaea have also been recently reported: *Sulfolobus solfataricus* [117], *Pyrodictium abyssi* [134,135] and a number of *Thermofilum* strains [136]. Crystal structure analyses, sequence alignments and mutagenesis studies have

indicated that mesophilic and thermophilic xylanases are very similar and that enhanced stability is probably due to an array of minor modifications, with many xylanases using unique strategies to improve their thermostability.

PSYCHROPHILES FOR XYLANASE PRODUCTION

Even though cold-temperature environments are the most abundant on earth [137], only a small number of cold-adapted, or psychrophilic, xylanase producers have been identified. These encompass a wide range of organisms; two gram negative bacteria [*Pseudoalteromonas haloplanktis* TAH3a [138-141] and *Flavobacterium frigidarium* sp. nov. [142], a gram positive bacterium [*Clostridium* strain PXYL1 [143], a yeast isolate [*Cryptococcus adeliae* [144], krill [*Euphasia superba* [145], a number of fungi [*Penicillium* sp., *Alternaria alternata* and *Phoma* sp. 2 [146] and a number of basidiomycetes [e.g., *Coprinus psychromorbidus* [147]. All have been isolated from the Antarctic environment, but, apart from the bacterial xylanase from *Pseudoalteromonas haloplanktis* TAH3a [pXyl] and the *Cryptococcus adeliae* xylanase [XB], studies of the xylanases produced are minimal. In accordance with most other psychrophilic enzymes investigated to date [148–150], the common features of the psychrophilic xylanases studied are a low temperature optimum, high catalytic activities at low temperatures and poor stability. To be sure, comparative studies of pXyl and XB with mesophilic xylanases showed that these enzymes have a higher catalytic activity at low and moderate temperatures, having, respectively, 10 and 3 times higher activity at 5°C and 3 and 2 times higher activity at 30°C [138]. Moreover, all psychrophilic enzymes studied display high catalytic activity at low temperatures. At 5°C, activity of pXyl is 60% of the maximum while xylanases from *Euphasia superba* display, respectively, approximately 30% and 40% of their maximum activity. In comparison, a mesophilic xylanase showed less than 5% of its maximum activity at this temperature [138].

ALKALIPHILES AND ACIDOPHILES FOR XYLANASE PRODUCTION

While the majority of natural environments on earth are essentially neutral, with pH values of between 5 and 9, habitats with extreme pHs are also common, in particular in geothermal regions, carbonate laden soils, soda deserts and soda lakes such as found in Egypt [Wadi Natrun], the African Rift valley [Lakes Magadi and Nakuru in Kenya], Central Asia, Western USA [Yellowstone National Park, USA] and Southern Europe [Vulcano Island, Italy]. Indeed, xylanase producing alkaliphilic micro-organisms, which typically grow optimally at pH values above 9, and acidophiles, which grow optimally between pH 1 and

5, have been isolated from these environments [151-153] and also from such sources as kraft pulp [154], pulp and paper industry wastes [155], decomposing organic matter [156], faeces [152], plant sources [157], soils [151,158] and even from neutral environments where they are found coexisting with neutrophilic micro-organisms [152]. The first report of a xylanase produced by an alkaliphilic micro-organism was as early as 1973 for a xylanase from *Bacillus sp.* C-59-2 [159] and since this initial finding a number of xylanases have been isolated from various acidophilic and alkaliphilic micro-organisms. These include xylanases from a number of *Bacillus sp.* [160] *Trichoderma sp.* [161,162,163], *Aspergillus sp.* [164,165], *Penicillium sp.* [151], *Acidobacterium sp.* [166] and *Cryptococcus sp.* [167]. Many of the alkaliphilic microorganisms studied have been found to produce xylanases with pH optima in the near neutral region but with relatively high activities being retained in alkaline conditions. In addition, a number of xylanases with more alkaline pH optima have also been isolated and one the most alkaliphilic xylanases reported to date is xylanase from *Bacillus sp.* AR-009, which has a pH optimum of pH 9–10 [153]. Other highly alkaliphilic xylanases include xylanase J from *Bacillus sp.* strain 41M-1 [158] and a xylanase from *Bacillus pumilus* 13a [156], both of which have a pH optimum of 9. Much fewer acidophilic than

alkaliphilic xylanases have been studied and the most important of these are the family 10 and 11 members from *T. reesei* [166] *A. niger* [165], *Cryptococcus sp.* S-2 [167], *Aspergillus kawachii* [168] and *Penicillium sp.* 40 [151]. Certainly, it was even suggested that adaptation to high pH may occur via a similar mechanism to adaptation to high temperatures [131].

INDUSTRIAL APPLICATIONS OF XYLANASE

Global markets for industrial enzymes grew from €1 billion in 1995 [169] to almost €2 billion in 2001 [170] and continue to increase as new enzymes and applications are discovered. In the grain-processing enzymes sector alone [which currently accounts for approximately 25–28% of total enzyme sales] an increase in market value from €510 million in 2001 to €760 million in 2010 has been forecasted [170].

Presently the technical industries, dominated by the detergent, starch, textile and fuel alcohol industries, account for the majority of the total enzymes market, with the feed and food enzymes together totaling only about 35%. Recently however, sales in some of the major technical industries has stagnated [3% drop in 2001] while sales in both the food and feed industries are increasing, with annual growth rates of approximately 4–5% being forecasted [170]. Various industrial uses of xylanase are shown in Fig 3.

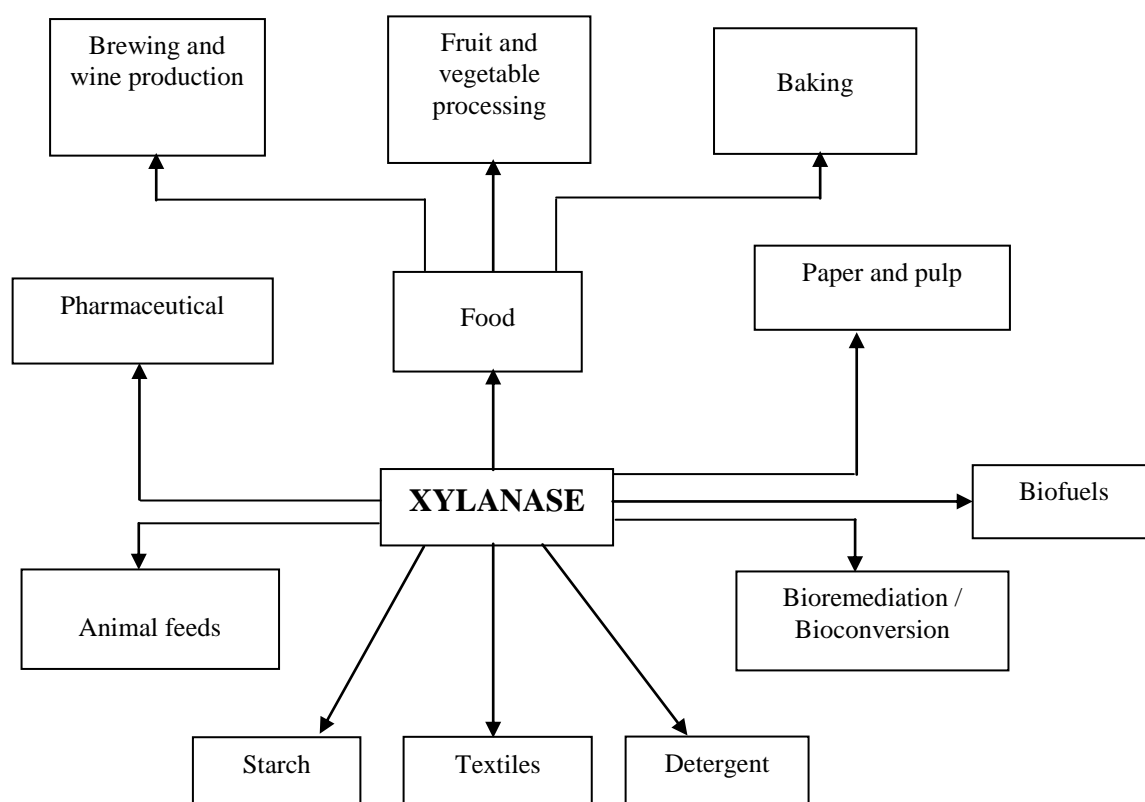


Fig.3. Various industrial applications of xylanase

PULP AND PAPER INDUSTRIES

The xylanase produced by the microorganisms and enzymes attacks hemicellulose and alters the interface between the cellulose and lignin, thereby facilitating the removal of the lignin-associated hemicellulosic fraction with minimal damage to the pulp. This process is less drastic, less expensive, and especially less toxic than conventional chemical treatment. Besides bleaching through lignin removal, the use of xylanases helps increase pulp fibrillation, reduce beating times in the original pulp, and increase the freeness in recycled fibers [171-173]. A treatment with xylanases can improve the chemical extraction of lignin from pulp. This leads to a significant reduction in the amount of chemicals required for bleaching and in the levels of toxic chlorine compounds released into the environment.

XYLANASES IN ANIMAL FEED

The use of enzymes in the production of feed is an important sector of agribusiness, with an annual world production exceeding 600 million tons and a turnover of >50 billion dollars. Xylanases are used in animal feed along with glucanases, pectinases, cellulases, proteases, amylases, phytase, galactosidases and lipases. These enzymes break down arabinoxylans in the ingredients of the feed, reducing the viscosity of the raw material [174]. The arabinoxylan found in the cell walls of grains has an anti-nutrient effect on poultry. When such components are present in soluble form, they may raise the viscosity of the ingested feed, interfering with the mobility and absorption of other components. If xylanase is added to feed containing maize and sorghum, both of which are low viscosity foods, it may improve the digestion of nutrients in the initial part of the digestive tract, resulting in a better use of energy. The joint action of the rest of the enzymes listed produces a more digestible food mixture. Young fowl and swine produce endogenous enzymes in smaller quantities than adults, so that food supplements containing exogenous enzymes should improve their performance as livestock. Moreover, this kind of diet is found to reduce unwanted residues in the excreta [phosphorus, nitrogen, copper and zinc], an effect that could have a role in reducing environmental contamination.

FOOD INDUSTRIES

Xylanases may be employed in bread-making, together with α -amylase, malting amylase, glucose oxidase and proteases. The xylanases, like the other hemicellulases, break down the hemicellulose in wheat-flour, helping in the redistribution of water and leaving the dough softer and easier to knead. During the bread-baking process, they delay crumb formation, allowing the dough to grow. With the use of xylanases, there has been an increase in bread volumes, greater absorption of water and improved resistance to fermentation

[175-177]. Also, a larger amount of arabinoxyloligosaccharides in bread would be beneficial to health. In biscuit-making, xylanase is recommended for making cream crackers lighter and improving the texture, palatability and uniformity of the wafers. The juice and wine industries make up a good part of the enzyme market. The production of fruit and vegetable juices requires methods of extraction, clearing and stabilization. In the 1930s, when the manufacture of citrus fruit juices began, the yields were low and problems were encountered in the filtration of the juice, owing to its turbidity [176]. The increase in knowledge of the chemical constituents of fruits and the use of microbial enzymes helped to solve these problems. Nowadays, xylanases, in conjunction with cellulases, amylases and pectinases, lead to an improved yield of juice by means of liquefaction of fruit and vegetables; stabilization of the fruit pulp; increased recovery of aromas, essential oils, vitamins, mineral salts, edible dyes, pigments etc., reduction of viscosity, hydrolysis of substances that hinder the physical or chemical clearing of the juice, or that may cause cloudiness in the concentrate [175]. Xylanase, in combination with endoglucanase, takes part in the hydrolysis of arabinoxylan and starch, separating and isolating the gluten from the starch in the wheat flour. This enzyme is also used in coffee-bean mucilage [178,179]. The main desirable properties for xylanases for use in the food industry are high stability and optimum activity at an acid pH.

PHARMACEUTICAL AND CHEMICAL INDUSTRIES

Xylanases are sometimes added in combination with a complex of enzymes [hemicellulases, proteases and others] as a dietary supplement or to treat poor digestion, but few medicinal products can be found with this formulation. Hydrolytic products of xylan, such as β -D-xylopyranosyl residues, can be converted into combustible liquids [ethanol], solvents and artificial low-calorie sweeteners. The first steps are the delignification of hemicellulose material rich in xylan, followed by hydrolysis by xylanases and hemicellulases, to produce sugars such as β -D-xylopyranosyl units. Next, the products are fermented, mainly by yeasts [*Pichia stipitis* and *Candida shehatae*], to produce xylitol or ethanol [180,181]. Among the sugars used in the production of ethyl alcohol, β -D-xylopyranosyl commercial xylitol is produced on a large scale by chemical catalysis. This is considered a high-cost process, mainly because the xylose has to be purified initially in several steps. Besides this, the chemical reactions often produce by-products toxic to fermentation; indeed, in the decomposition of lignocellulosic material, besides the liberation of sugars, products may be formed that are derived from the degradation of glucose [hydroxymethylfurfural], xylose [furfural] and lignin

[aromatic and phenolic compounds and aldehydes] [180]. Substances liberated from the lignocellulose structure, such as acetic acid and extracted material [e.g. terpenes and their derivatives, tropolones and phenolic compounds such as flavonoids, stilbenes, quinones, lignans and tannins], or from the equipment [iron, chromium, nickel and copper], can be powerful inhibitors of microbial activity [181]. The development of a more appropriate technology for xylitol production has generated great hope of its wider use in the food, pharmaceutical and odontological industries. Residues represent between 5 and 20%. Xylitol is a polyalcohol with a sweetening power comparable to that of sucrose [182]. It is a non-cariogenic sweetener, suitable for diabetic and obese individuals and recommended for the prevention of osteoporosis and respiratory infections, lipid metabolism disorder, kidney and parenteral lesions. A variety of commercial products containing xylitol, such as chewing gum, can be found on the market. Although the enzymatic hydrolysis of xylan is a promising method of obtaining β -D-xylopyranosyl units, at present.

TEXTILE

The xylanolytic complex can be used in the textile industry to process plant fibres, such as hessian or linen. For this purpose, the xylanase should be free of cellulolytic enzymes. One process consists of incubating dried ramee [China grass] stems with xylanase to liberate the long cellulose fibres intact. After using this method, there is no need to use the strong bleaching step, since the lignin does not undergo oxidation, which would lead to darkening of the fibres [183-185]. Relatively little research has been done on the enzymatic preparation of textile fibres, and yet this appears to be a promising market demanding the development of new techniques.

CONCLUSIONS

Xylanases are produced by a large number of different microorganisms. These organisms differ greatly with respect to xylanase activities and properties attained. As discussed here, xylanases are multipurpose enzymes that are used widely. xylanases are becoming increasingly important in high-value applications in pulp and paper industries and so many other industries. Biobleaching process needs xylanase that are active at high temperature and pH. The microorganisms produced xylanase with high temperature and pH optima, thereby suggesting its potential in biobleaching processes. When the xylanase enzyme was used for biobleaching process, a considerable amount of reduction in kappa number of the kraft pulp without much change in viscosity was observed. Hence, there was reduction in the amount of chlorine during the bleaching process. Moreover, the enzyme treatment enhanced the brightness of the pulp. Xylanases with improved properties are being produced by natural

selection and protein engineering to further enhance usefulness of these enzymes.

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